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Chemical composition and nutritional evaluation of protein feeds for ruminants using an *in vitro* gas production technique

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The nutritive value of nine protein feeds were evaluated by an *in vitro* gas production technique. The rumen mixed microbe inoculum source was taken from two fistulated Brahman-Thai native crossbred steers. The protein feed sources were soybean meal, coconut meal, palm meal, soybean hull (pellet), full fat soybean, dried brewer's grain, coconut meal (coconut milk press), kapok seed and leuceana meal. The results showed that the soluble gas fraction (a) was -1.21, -0.90, -0.55, -12.18, -2.67, -1.24, -2.15, -4.73 and -2.18 ml, respectively; the fermentation of insoluble fraction (b) was 105.25, 63.25, 90.83, 160.65, 98.48, 95.93, 70.32, 33.88 and 62.47 ml, respectively; the rate of gas production (c) was 0.033, 0.044, 0.024, 0.042, 0.034, 0.015, 0.020, 0.050 and 0.026 %/hr respectively; the potential of extent of gas production ($|a|+b$) was 107.32, 64.89, 94.36, 172.83, 101.66, 97.32, 73.19, 38.61 and 65.28 ml, respectively, and the estimated metabolizable energy (ME) was 8.10, 4.97, 4.69, 5.61, 7.10, 5.39, 3.51, 5.088 and 4.47 MJ/kg DM, respectively and were significantly different (P<0.01) among protein feed sources. The cumulative gas volume at 24, 48 and 96 hr after incubation were significantly different (P<0.01). Soybean meal, soybean hull (pellet) and full fat soybean showed the highest *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) soybean meal was the highest for estimated metabolic energy. These results suggest that soybean meal, soybean hull (pellet) and full fat soybean are high potential protein feed sources for ruminant feeds.

Key words: *in vitro* gas production, protein feeds, ruminant

Introduction

The nutritive value of a feed is assessed by amount of nutrients containing chemical composition, digestibility and level of voluntary feed (Ibrahim *et al.*, 1995). Feed evaluation methods are use to express nutritive

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value of feed. It is basically description of feeds interns that allow for a prediction of the performance of animals offered the feeds (Medsen *et al*., 1997). There are many methods used in feed evaluation such as chemical analysis, degradability measurement, digestibility measurement and feed intake prediction. The in vitro gas production technique has proved to be a potentially useful technique for feed evaluation (Herrero *et al*., 1996; Getachew *et al*., 2004), as it is capable of measuring rate and extent of nutrient degradation (Groot *et al*., 1996; Cone *et al*., 1996). In spite of numerous studies conducted on the used of protein as ruminant feed, limited information is available on the kinetics of digestion and metabolizable energy for ruminant using the *in vitro* gas production technique and also little research has characterized individual feeds.

With respect to protein feed sources in Thailand, limited information is available on kinetics of gas production. The aim of this study was, therefore, to evaluated nutritive values of protein feed sources in ruminants using the *in vitro* gas production technique.

Materials and methods

Feedstuffs samples and chemical analysis

The protein feeds were collected from various feed mills and organizations in the North East of Thailand. All samples were ground through a 1 mm screen for the in vitro gas production technique incubation and chemical analysis. The samples were determined for dry matter (DM), crude protein (CP) and ash content (AOAC, 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) of samples were assayed using the method proposed by Van Soest *et al*. (1991).Concentrations of Ca, Mg, K, Na, Mn, Cu and Fe of feedstuffs were determined by Atomic Absorption Spectrophotometer (AA 680, Shimadzu, Japan).

Experimental design and gas production technique

The experimental design was completely randomized with eight replicates per treatment. The treatments included soybean meal, coconut meal, palm meal, soybean hull)pellet(, full fat soybean, dried brewer’s grain, coconut meal)coconut milk press(, kapok seed and leuceana meal. Strict anaerobic techniques were used in all steps during the rumen fluid transfer and incubation period. Rumen fluid inoculum was removed before the morning feeding under vacuum pressure via the rumen fistula into a 2 liter glass flask and transferred into two pre-warmed 1 liter thermos flasks which were then transported to the laboratory. The medium preparation was as described by Makkar *et al*., (1995). Mixed rumen fluid inoculums were obtained from two fistulated Brahman-Thai native crossbred steers (weighed 250+15 kg). The animals were offered rice straw *ad libitum* and 0.5 % body weight of concentrate. The animals were fed twice daily, water and a mineral lick were available *ad libitum* for 14 days.

The feed sample of approximately 0.5 g on a fresh weight basis was transferred into a 50 ml serum bottle (Sommart *et al*., 2000). The bottles were pre-warmed in a hot air oven at 39 °C for about 1 hour prior to injection of 40 ml of rumen fluid medium (using a 60 ml syringe) to each bottle. The bottles were stoppered with rubbers stoppers, crimp sealed and incubated in a hot air oven set at 39 °C.

The rate of gas production was measured by reading and recording the amount of gas volume after incubation using a 20 ml glass syringe connected to the incubation bottle with a 23 gauge, 1.5 inch needle. Readings of gas production were recorded from 1 to 96 hr (hourly from 1-12 hr, every 3 hr from 13-24 hr, every 6 hr from 25-48 hr and every 12 hr from 49-96 hr) after incubation periods. Amount of cumulative gas volume at 2, 4, 6, 12, 24, 48, 72 and 96 hr after incubations were fitted using the equation $y= a+b\ [(1-\mathrm{Exp}(-ct)]$ (Ørskov and McDonald, 1979), where a is the intercept, which ideally reflects the fermentation of the soluble fraction, b is the fermentation of the insoluble fraction, c is rate of gas production, $(a+b)$ is potential extent of gas production, y is gas production at time ‘t’.

In vitro digestibility of dry matter and organic matter were measured at 24 and 96 hr after incubation. The metabolizable energy were calculated as ME, MJ/kgDM = 2.20 + (0.136xGv) + (0.057x%CP), Menke *et al.* (1979), where Gv = gas volume at 24 hr, CP=%crude protein in feedstuffs.

Statistical analyses

All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a completely randomized design. Means were evaluated by Duncan New’s Multiple Range Test. The level of significance was determined at $P<0.05$.

Results and discussion

Chemical compositions and mineral content of protein feed sources

The chemical compositions and mineral content of protein feed sources are presented in Table 1. Generally, wide variations existed in the chemical composition of the investigated feedstuffs. The crude protein content ranged from 4.77 % for coconut meal)coconut milk press) to 49.56 % for soybean meal. Ash content ranged from 1.08 % for coconut meal to 17.69 % for leuceana meal. NDF content ranged from 13.29 % for soybean meal to 70.90 % for soybean hull (pellet). ADF ranged from 9.48 % for soybean meal to 45.65 % for leuceana meal. Acid detergent lignin ranged from 1.28 % for soybean meal to 22.30 % for kapok seed.

This study indicates that soybean hull (pellet) was the highest in Ca content as compared to the other protein feed source. Dried brewer’s grain was shown to have the lowest Ca content. However, Dried brewer’s grain had the highest P content. There are many factors that affect chemical composition and mineral content of feedstuffs such as oil extraction process (Mara *et al*., 1999), stage of growth maturity, species or variety (von Keyserlingk *et al*., 1996; Agbagla-Dohnani *et al*., 2001 and Promkot and Wanapat, 2004), drying method, growth environment, (Mupangwa *et al*., 1997) and soil types (Thu and Preston, 1999). These factors may partially explain differences in chemical composition between our study and others.

Gas production characteristics of protein feeds

Gas production in the fermentation of protein feed sources were measured at 2, 4, 6, 12, 24, 48, 72 and 96 hr using *in vitro* gas production technique adapted to describe the kinetics of fermentation based on the modified exponential model y= $a+b$ [(1-Exp(-ct)] (Ørskov and McDonald, 1979). Although there are other models available to describe the kinetics of gas production, the Ørskov and McDonald (1979) model was chosen because the relationship of its parameters with intake, digestibility and degradation characteristic of forages and concentrate feedstuffs had been documented (Blummel and Ørskov,1993; Khazaal *et al*., 1993; Sommart *et al*., 2000; Nitipot and Sommart, 2003).

Gas production characteristics are presented in Table 2. A comparison of gas production characteristics of different treatments indicated significantly differences (between them) (P<0.01). The value for a intercept for all feeds

ranged from –12.18 to -0.55 ml. Palm meal had the lowest value for *a*, intercept, while whole soybean hull had the highest value for it. The values for *a* intercept were negative in the incubations in this study. These data suggested that a lag phase due to delay in microbial colonization of the substrate may occur in the early stage of incubation. Several authors (Khazaal *et al*., 1993; Blummel and Becker, 1997) have also reported negative values with various substrates when mathematical models was applied to fit gas production kinetics. This is due to either a deviation from the exponential cause of fermentation or delays in the onset of fermentation due to the microbial colonization. It is well known that the value for absolute *a* (|*a*|), intercept ideally reflect the fermentation of the soluble fraction. In this study the |*a*| was the highest for soybean hull. The soluble fraction in soybean hull was also found to be the highest among the feeds.

The gas volume at asymptote (*b*) described the fermentation of the insoluble fraction. The gas volumes at asymptote of soybean meal, coconut meal, palm meal, soybean hull)pellet(, full fat soybean, dried brewer's grain, coconut meal)coconut milk press(, kapok seed and leuceana meal were as follows:- 105.25, 63.25, 90.83,160.65, 98.48, 95.93, 70.32, 33.88 and 62.47 ml, repetitively. It can be demonstrated that gas production at asymptote of kapok seed, leuceana meal and palm meal were very low when compared to the other feeds, possibly reflecting high level of lignin (Table 1) (Chumpawadee *et al*., 2005a). In addition, kapok seed and leuceana meal have an anti-nutritional compounds, such as cyclopropenoid fatty acid and mimosin, which are toxic to rumen microbe. The feedstuffs has a high value of undegraded protein, also leading to difficult attachment by microorganisms (NRC, 2001). The high fermentation of the insoluble fraction were observed in soybean hull, possibly influenced by the carbohydrate fractions readily available to the microbial population.

Rates of gas production (*c*) expressed in percent/hr, ranked from the fastest to the slowest, were kapok seed, coconut meal, soybean hull (pellet), full fat soybean, soybean meal, leuceana meal, palm meal, coconut meal (coconut milk press) and dried brewer's grain. Potential extent of gas production (|*a*|+*b*) expressed in ml as ranked from the highest to lowest which were soybean hull (pellet), soybean meal, full fat soybean, dried brewer's grain, palm meal, coconut meal (coconut milk press), leuceana meal, coconut meal and kapok seed. Remarkably, the potential of gas production for protein feed sources was lower than that of carbonaceous concentrates feedstuffs (Chumpawadee *et al*., 2005b). The results was in agreement with the report of Gatachew *et al*. (1998), who suggested that gas production is basically the result of the fermentation of carbohydrates into acetate, propionate and butyrate. Khazaal *et al*. (1995) also reported that protein fermentation does not lead to extensive gas production. In this study, high potential extents of gas production were observed in soybean hull, soybean meal, full fat soybean, while the potential extent of gas production in coconut meal (coconut milk press), leuceana meal, coconut meal and kapok seed which were low. This implies that soybean hull, soybean meal and full fat soybean were highly ferment able in the rumen.

Gas volume of protein feeds

The cumulative gas volumes at 24, 48 and 96 hr after incubation are shown in Table 2. The results were significantly different (P<0.01) between treatments. Based on these observations of protein feed sources, the gas volumes ranked from the highest to the lowest which were soybean hull (pellet), soybean meal, full fat soybean, dried brewer’s grain, palm meal, coconut meal (coconut milk press), leuceana meal, coconut meal and kapok seed. Cumulative gas volume at each sampling time was affected by a variety of protein feed sources. These findings indicate that the fraction of substrate and degradability of protein feed sources are different. Gas is produced directly proportional to the rate of substrate degradation (Dhanoa *et al*., 2000). Additionally, kinetics of gas production is dependent on the relative proportions of soluble, insoluble but degraded, and undegradable particles of the feed (Getachew *et al*., 1998). Menke *et al*. (1979) suggested that gas volume at 24 hr after incubation has a direct relationship with metabolizable energy level in feedstuffs. Sommart *et al*. (2000) reported that gas volume is a good parameter to predict digestibility, volatile fatty acids production and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Additionally, *in vitro* dry matter and organic matter digestibility were shown to be a high correlation with gas volume (Nitipot and Sommart, 2003). Gas volume also has related to a correlation with feed intake (Blummel and Becker, 1997) and growth rate)Blummel and ∅rskov, 1993).

In vitro dry mater and organic matter digestibility

In vitro dry mater and organic matter digestibility at 24 and 96 hr after incubation are shown in Table 2. It can be seen that *in vitro* dry mater and organic matter digestibility are in the same way. The *in vitro* dry matter and organic matter digestibility at 24 and 96 hr after incubation significantly differ among the tested protein feeds (P<0.01). High digestibility of dry matter and

organic matter at 96 hr were observed in soybean meal, soybean hull and full fat soy bean. This result implies that the microbe in the rumen and animal had high nutrient uptake. The higher fiber content (Table 1) of kapok meal, leuceana meal, coconut meal and coconut meal (Coconut milk press) probably resulted to lower *in vitro* dry matter and organic matter digestibility when high NDF and ADL content in feedstuffs which resulted to lower fiber degradation (Van Soest, 1988). In general, the tropical forages and concentrate feedstuffs have a large proportion of lignified cell walls with low fermentation rates and digestibility, leading to low digestibility rates and limited intake (Ibrahim *et al*., 1995).

Estimated metabolizable energy (ME)

Metabolizable energy predicted by the equation of Menke *et al*. (1979) is as follows: $\mathrm{ME,\ MJ/kgDM} = 2.20 + (0.136\mathrm{x}Gv) + (0.057\mathrm{x}\%CP)$ where Gv is gas volume at 24 hr) ml), CP iscrude protein in feedstuff) %). The ME value of protein feeds are shown in Table 2. Soybean meal had the highest metabolizable energy and different from other protein feed souces. Menke and Steingass (1988) reported a strong correlation between metabilizable energy (ME) values measured *in vivo,* (predicted from) 24 hr *in vitro* gas production and chemical composition of feed. The *in vitro* gas production method has also been widely used to evaluate the energy value of several classes of feed (Getachew *et al*., 1998; Getachew *et al*., 2002). Krishnamoothy *et al*. (1995) also suggested *in vitro* gas production technique should be considered for estimated metabolizable energy in tropical feedstuff because evaluation of ME by other technique required labor, cost, time and complexity.

Conclusions

The protein feed sources showed a great variation in chemical composition and mineral content. The results of this study demonstrates that kinetics of gas production of protein feed sources differed among feed. Based on this study, high ferment abilities for protein feeds used in ruminant ranked from the highest to the lowest were soybean hull (pellet), soybean meal, full fat soybean, Dried brewer’s grain, palm meal, coconut meal (coconut milk press), leuceana meal, coconut meal and kapok seed, respectively.

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Table1. Chemical composition and mineral content of protein feeds.

Feedstuffs	DM (%)	CP	Ash	NDF	ADF	ADL	Ca	P	Mg	K	Na	Mn	Cu	Fe
		%DM basis					mg/gDM							
Soybean meal	92.86	49.56	7.57	13.29	9.48	1.28	3.83	4.22	1.81	16.00	2.90	0.031	0.033	0.164
Coconut meal	95.45	9.94	4.86	53.09	34.48	7.83	2.01	2.60	0.97	8.84	2.34	0.035	0.011	0.198
Palm meal	95.36	8.79	5.17	60.92	44.48	20.46	0.20	5.30	1.63	8.61	0.64	0.121	0.019	0.266
Soybean hull (pellet)	92.57	12.18	5.71	70.90	41.83	2.49	8.58	2.07	1.19	11.41	0.55	0.009	0.006	0.272
Full fat soybean	93.47	38.87	7.00	17.05	9.53	1.47	0.48	6.25	1.42	13.23	0.49	0.013	0.009	0.109
Dried brewer’s grain	92.45	29.13	5.72	60.11	20.38	5.72	0.06	9.30	1.14	1.07	3.81	0.063	0.010	0.255
Coconut meal (Coconut milk press)	93.28	4.77	1.08	69.10	33.59	6.45	0.12	1.19	0.60	3.88	1.21	0.016	0.012	0.066
Kapok seed	92.76	24.01	1.32	40.41	28.79	22.30	0.50	5.23	1.25	6.20	0.26	0.016	0.012	0.069
Leuceana meal	92.49	10.36	17.69	59.49	45.65	11.66	0.13	3.46	1.49	10.71	3.10	0.065	0.006	0.473

Table 2. Gas production characteristics, gas volume and estimated metabolizable energy of protein feeds using *in vitro* gas production technique

Parameters	Feedstuffs									SEM
	SB	**CM**	**PM**	**SHP**	**FSB**	**DBG**	**CMP**	**KS**	**LM**	
Gas production characteristic parameters										
a, ml	-1.21 a	-0.90 a	-0.55 a	-12.18 c	-2.67 ab	-1.24 a	-2.15 ab	-4.73 b	-2.81 ab	0.41
b, ml	105.25 b	63.25 d	90.83 bcd	160.65 a	98.48 bc	95.93 bc	70.32 cd	33.88 e	62.47 d	3.97
c, %/hr	0.033 dc	0.044 ab	0.024 def	0.042 abc	0.034 bcd	0.015 f	0.020 ef	0.050 a	0.026 de	0.00
$\|a\|+b$, ml	107.32 b	64.89 de	94.36 bc	172.83 a	101.66 bc	97.32 bc	73.19 cd	38.61 e	65.28 de	4.13
In vitro digestibility, %										
IVDMD,24 hr	78.87 a	25.59 cd	31.16 c	24.79 cd	57.42 b	24.93 cd	18.05 d	55.20 b	14.88 d	2.42
IVOMD, 24 hr	79.45 a	27.76 cd	32.55 c	25.89 cd	57.55 b	26.20 cd	20.80 de	57.35 b	12.56 e	2.37
IVDMD, 96 hr	98.80 a	56.83 c	70.91 b	96.16 a	95.19 a	69.88 b	37.77 d	58.31 c	48.36 c	2.41
IVOMD, 96 hr	98.93 a	57.23 c	70.78 b	96.32 a	95.03 a	70.59 b	40.26 d	59.91 bc	46.18 d	2.35
Gas volume(ml/0.5gDM)										
GV,24 hr	56.60 a	40.44 b	36.50 bc	49.90 a	52.77 a	28.20 cd	19.17 d	27.70 cd	30.81 c	1.54
GV,48 hr	83.37 b	52.44 de	64.23 cd	105.70 a	73.40 bc	51.45 de	30.43 f	34.10 f	47.11 e	2.52
GV,96 hr	101.50 b	63.50 e	80.30 cd	152.80 a	89.17 bc	72.00 de	41.67 f	39.80 f	60.31 e	3.30
ME, Mj/kgDM	8.10 a	4.97 def	4.69 ef	5.61 c	7.10 b	5.39 cd	3.51 g	5.08 de	4.47 f	0.15

Note: SB =Soybean meal, CM= Coconut meal, PM= Palm meal, SHP= Soybean hull (pellet), FSB= Full fat soybean, DBG= Dried brewer’s grain, CMP= Coconut meal (Coconut milk press), KS= Kapok seed, LM = Leuceana meal, a = describe ideally reflects the fermentation of the soluble fraction, b= described the fermentation of the insoluble fraction, c= Rates of gas production, $|a|+b$= Potential extent of gas production, IVDMD= in vitro dry matter digestibility, IVOMD= in vitro organic matter digestibility, GV= Gas volume